REMARKS

Claims 46, 48-53, 58, 63, 65, and 66 have been amended by way of this amendment. Claims 31, 33-42, 44, 45, 47, 54-57, 61-62 have been cancelled, without prejudice or disclaimer, by way of this amendment. Claims 67-75 have been added by way of this amendment. The Examiner has indicated that claim 43 appears to be in condition for allowance. Claims 43, 46, 48-53, 58-60, 63-75 (of which 49-53 and 60 are currently withdrawn) remain pending in this application.

Claims 46, 48-53, and 63 have been amended to correct grammatical informalities (e.g. to recite "a method" rather than "the method"). In addition, claim 50 was amended to correct the spelling of the term "pyrimidine." Claims 46, 48, 49, and 58 have been amended to change the claim from which they depend and to make proper reference to the subject matter of the claim from which they now depend. No new matter has been added by way of these amendments.

Claim 63 has been amended to recite specific *udp* and *deoD* nucleotide sequences. This is supported by page 5, lines 8-11 of the specification and, for example, the descriptions in the sequence listing filed on June 25, 2001 of SEQ ID NO: 6, where it is stated that nucleotides 243 to 1021 correspond to the *udp* gene and that nucleotides 1037 to 1766 correspond to the *deoD* gene. Claims 65 and 66 have been amended to make proper reference to the subject matter of the claim from which they depend (claim 63; which is directed to a host cell). Accordingly, these claims have been amended to be directed to "a host cell" rather than "a plasmid vector." No new matter has been added by way of these amendments.

Support for new claim 67 can be found in originally filed claim 4 of PCT/EP99/10416 (the PCT application of which the instant application is a continuation application; herein "the '416 PCT application") and throughout the specification and, in particular, on page 5, lines 21-24; and page 7,

line 8 - page 9, line 18. Support for new claim 68 can be found in originally filed claim 10 of the '416 PCT application and throughout the specification and, in particular, on page 7, lines 4-5. Support for new claim 69 can be found in originally filed claim 11 of the '416 PCT application and throughout the specification and, in particular, on page 7, line 5. Support for new claim 70 can be found in originally filed claim 28 of the '416 PCT application and throughout the specification and, in particular, on page 6, lines 11-15.

New claims 71-75 depend from withdrawn claims 49, 51, and 52; the withdrawn claims from which they depend have been amended by way of the instant amendment to put their language into proper claim form. New claims 71-75 have been added to provide claims that are directed to the subject matter of claims 49, 51, and 52 that has been deleted from these claims by way of this amendment. The withdrawn claims are being amended to be put into proper form because the Examiner has acknowledged that if a product claim is found allowable, withdrawn process depending from or otherwise including all of the limitations of the allowable product claim will be rejoined in accordance with MPEP § 821.04. No new matter has been added by way of these new claims and Applicants respectfully request their consideration.

Support for new claim 71 can be found in originally filed claim 23 of the '416 PCT application and throughout the specification and, in particular, on page 13, line 28 - page 14, line 6. Support for new claim 72 can be found in originally filed claim 22 of the '416 PCT application and throughout the specification and, in particular, on page 13, line 29 - page 14, line 1. Support for new claim 73 can be found in originally filed claim 22 of the '416 PCT application and throughout the specification and, in particular, on page 14, line 1-6. Support for new claim 74 can be found in originally filed claim 24 of the '416 PCT application and throughout the specification and, in

particular, on page 13, line 24. Support for new claim 75 can be found in originally filed claim 24 and throughout the specification and, in particular, on page 13, lines 21-28. No new matter has been added by way of these new claims.

Priority

The Examiner has stated that foreign priority document MI 98 A 002792 has not been received by the Office and that the conditions of 35 U.S.C. § 119(b) have not been satisfied. Applicants respectfully note that MI 98 A 002792 is the application number of Italian patent number IT 1304500. As the Examiner has acknowledged in paragraph 13 (page 3) of the June 30, 2004 Action, IT 1304500 (and, thus, MI 98 002792) has been received by the USPTO and foreign priority under 35 U.S.C. § 119(a)-(d) has been acknowledged. Accordingly, no additional certified copies of foreign priority documents should be required and it is respectfully requested that the Examiner acknowledge that the foreign priority document (there is only one) has been received and that the conditions of 35 U.S.C. § 119(b) has been satisfied.

Specification/Informalities

The Examiner has objected to the title of the application for allegedly not being descriptive. The Examiner has suggested that the term "nucleotide" in the present title be replaced by the term "nucleoside." As suggested by the Examiner, the title has been changed by way of this Response and Amendment to: "Vectors, Host Cells, and Methods for Production of Uridine Phosphorylase and Purine Nucleoside Phosphorylase."

Claim Objections

Claim 31 has been objected to for recitation of "/or" in the claim. Claim 31 has been cancelled by way of this amendment. Recitation of "/or" in claims 50, 51, and 63 has been deleted. Accordingly, it is believed this rejection has been obviated and its withdrawal is respectfully requested.

Claim 48 has been objected to as grammatically incorrect because of recitation of "[m]ethod of producing." Claim 48 (as well as withdrawn claims 49, 50, 51, 52, and 53) has been amended, as suggested by the Examiner, to recite "A method of producing." Accordingly, it is believed this rejection has been obviated and its withdrawal is respectfully requested.

Claim 61 has been objected to as reciting "[a] prokaryotic host cell according to claim 44" because claim 44 is drawn to a plurality of host cells. Accordingly, it is believed this rejection has is moot and its withdrawal is respectfully requested.

The Examiner has also objected to claims 63-64 as being substantial duplicates of claims 61-62, respectively. As claims 61-62 have been cancelled, without prejudice or disclaimer, withdrawal of this objection is respectfully requested.

Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 61–62 and 65–66 have been rejected by the Examiner for alleged indefiniteness. The Examiner contends there is an insufficient antecedent basis for the phrase "the prokaryotic host cell not containing the plasmid" in claim 61 (which claim 62 depends). As claims 61 and 62 have been cancelled, without prejudice or disclaimer, by way of this amendment it is believed this rejection is moot and its withdrawal is respectfully requested.

The Examiner contends that claims 65–66, which depend from claim 63, are confusing because they refer to "a plasmid vector according to claim 63," whereas claim 63 is directed to "a transformed prokaryotic host cell." Claims 65 and 66 have been amended to be directed to "A host cell according to claim 63..." Accordingly, it is believed this rejection has been obviated and its withdrawal is respectfully requested.

Rejections under 35 U.S.C. § 112, First Paragraph: Written Description

Claims 31, 33–42, 44–48, 58–59, and 61–66 have been rejected for alleged failure to fulfill the written description requirement. The Examiner states that the specification allegedly does not adequately describe the genus of proteins with UdP or PNP activity and that the specification allegedly only describes the genus by functional features.

Claims 31, 33-42, 44-45, 47, and 61-62 have been cancelled, without prejudice or disclaimer, by way of this amendment. Each of the claims subject to this rejection that is still pending in the instant application (claims 46, 48, and 58-59) depends, either directly or indirectly, from independent claim 63. Without conceding the Examiner's position, claim 63 has been amended to recite the particular nucleotide sequences that code for the UdP and PNP activity (specifically, nucleotides 243 to 1021 of SEQ ID NO: 6 for the UdP activity and nucleotides 1037 to 1766 of SEQ ID NO: 6 for the PNP activity). Accordingly, it is believed this rejection has been obviated and its withdrawal is respectfully requested.

Rejections under 35 U.S.C. § 112, First Paragraph: Enablement

Claims 31, 33–42, 44–48, 58–59, and 61–66 have been rejected for alleged failure to fulfill the enablement requirement. The Examiner acknowledges that the specification enables an expression vector comprising specific *udp* and *deoD* nucleotide sequences. However, the Examiner contends that the specification does not enable the complete genus of the UdP or PNP functional family of genes because the claims encompass any genes coding for proteins with UdP or PNP activities from any type of mesophilic organism.

Claims 31, 33-42, 44-45, 47, and 61-62 have been cancelled, without prejudice or disclaimer, by way of this amendment. Each of the claims subject to this rejection that is still pending in the instant application (claims 46, 48, and 58-59) depends, either directly or indirectly, from independent claim 63. Without conceding the Examiner's position, claim 63 has been amended to recite the particular nucleotide sequences that code for the UdP and PNP activity (specifically, nucleotides 243 to 1021 of SEQ ID NO: 6 for the UdP activity and nucleotides 1037 to 1766 of SEQ ID NO: 6 for the PNP activity). Accordingly, it is believed this rejection has been obviated and its withdrawal is respectfully requested.

Rejections under 35 U.S.C. § 103(a)

The Examiner has again rejected claims 31, 34–38, 40–42, 44–48, 58–59, and 61–66 as being allegedly obvious over Krenitsky[1] in view of Walton[2], Hershfield, Bulow[3], and Novagen[4].

[1] Krenitsky *et al.* U.S. Patent 4,347,315
[2] Walton *et al.* (1989) *Nucleic Acids Res.* **17**:6741
[3] Bulow *et al.* (1991) *Trends Biotech* **9**:226–31
[4] 1997 Catalog

Also, the rejection of claims 31, 34–39, 41–42, 44–45, 47–48, and 61-66 as being allegedly obvious over Krenitsky in view of Walton, Hershfield, Bulow, and Sambrook[5] has been maintained.

These rejections are respectfully traversed. In contrast to the Examiner's reasoning, the claims are unobvious over both combinations of references cited in the office action. The main reasons for the failure of these reference combinations to render the claims obvious are that (1) the primary reference, Krenitsky, does not obtain high enzyme activity in its production system and in fact teaches away from the present invention; and (2) the claims contain a material limitation not taught in the art.

- ***Krenitsky Teaches Away From the Claimed Invention***

The Examiner's attention is again directed to the following passage of Krenitsky (col. 4, lines 30-43):

> It has been found that crude enzyme preparations are less suitable than purified preparations. This is due to the fact that crude preparations contain troublesome nucleic acids as well as enzymes other than those required for the process of the present invention. The extraneous enzymes in crude preparations catalyse undesirable alterations of substrates and products, and may even cause proteolysis of the required enzymes themselves. These factors decrease not only the yield of the desired products but also the ease with which they can be isolated from reaction mixtures.
>
> In most cases therefore, it is desirable to purify the crude enzyme preparations before addition to the reaction mixture.

Accordingly, Krenitsky unequivocally teaches that in order to achieve high enzyme activity, "crude" enzyme preparations must be purified so that the enzymes are <u>isolated from other</u>

[5] Sambrook *et al.* (1989) "Molecular Cloning, A Laboratory Manual, Second Edition", Cold Spring harbor Press

components present in the environment in which they naturally occur, *i.e.*, a cell. In fact, Krenitsky teaches that the cellular components may interfere with enzyme activity ("crude preparations catalyse undesirable alterations of substrates and products, and may even cause proteolysis of the required enzymes themselves"). Thus, Krenitsky teaches that in order to get high enzyme activity, the enzymes must be purified from their cellular environment. This is in direct contrast to the presently pending claims that are directed to "[a] transformed prokaryotic host cell expressing 120-1000 times higher uridine phosphorylase activity, purine nucleoside phosphorylase activity or both, than the corresponding non-transformed prokaryotic host cell." Thus, the enzyme, as expressed, has higher activity before and without any need for purification.

The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990); (MPEP 2143.01). In the instant case, when considering Krenitsky as a whole, it cannot be combined with the other references, as suggested by the Examiner, to arrive at transformed host cell expressing 120-1000 times higher uridine phosphorylase activity, because it teaches away from the present invention.

Implicit in Krenitsky is an admission that as expressed, the enzyme activities do not have high activity. If they did, Krenitsky would not be so concerned with loss of such activity as to provide the forgoing forceful negative teaching.

- ***The Claimed Invention Requires "As Expressed" Enzyme Activities Not Taught by the Art***

As discussed above, because of its teachings as a whole, Krenitsky cannot be combined with the other references. For this reason alone, the claims are unobvious over the combinations of references suggested by the Examiner. However, even when forcefully combining Krenitsky with the references proposed by the Examiner, the resulting combination does not achieve the results seen by the inventors with the presently claimed cells.

In the Action the Examiner states (page 15, second-to last line - page 16, line 7):

> [o]ne would have a reasonable expectation of success that, by using the pET29c vector [taught in Novagen 1997] for expression of genes encoding E. coli UDP and PNP, a high yield of expression would be obtained. In this regard, the Office does not have the facilities for examining and comparing applicants' UDP and/or PNP activity in the host cells of claims 61-66 with that of the prior art. Thus, the burden is on the applicant to show a novel or nonobvious difference between the claimed product and the product of the prior art (i.e. that the level of UDP and PNP activity as expressed in an E. coli host using the pET29C vector does not possess the same material characteristics of the claimed host cell.) See *In re Best*, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and *In re Fitzgerald* et al. 205 USPQ 594.

As discussed in detail below, the presently claimed invention is directed to host cells expressing UdP and PNP activity levels that were surprising over those reported in the art. The high level of enzyme activity achieved in these cells is demonstrated in the specification in the following passage (page 12, first full paragraph):

> The surprisingly high level of enzyme activity of these novel recombinant strains is confirmed by an indirect comparison with the strains described in JP-06-253854: the strains considered in the present invention permit enzyme activities from 340 to 1040 times (as regards the activity of UdP) and from 120 to 200 times (as regards the

> activity of PNP) higher than the enzyme activities of the non-transformed wild-type strains; the strains described in JP-06-253 854, on the other hand, have an enzyme activity in E. coli 150 and 91 times higher, respectively, than that of the corresponding wild-type strain. It is also noteworthy that the enzyme activity of the strains of the present invention was determined at 30 °C, while that of the strains of JP-06-253854 was established while operating at 70 °C, or at a temperature which permits markedly higher kinetics.

Krenitsky states that "[f]or the purposes of the present invention ... aerobic bacteria such as *B. stearothermophilus* and especially *E. coli* B ... were found to be excellent sources of such enzymes" (column 4, lines 18-23), thereby teaching that in an *in vitro* setting, thermophilic (*B. stearothermophilus*) and mesophilic (*E. coli*) UdP and PNP enzymes are both effective. However, as reported in Noguchi (JP-06-253854), bacterial plasmids containing the gene sequences for UdP and PNP from thermophilic *B. stearmophilus* only yielded a 6-8-fold improvement of productivity as compared to one of the non-transformed control strain (page 31, last paragraph of Noguchi). The Examiner is incorrect in stating on page 15 of the Action that Noguchi (JP-06-253854) is silent as to the level of UDP and PNP overexpression in *E. coli*. The UDP and PNP levels reported in Noguchi are from *E. coli* expressing these enzymes (see e.g. page 30, line 5 of Noguchi, all of Example 3 of Noguchi, and page 24, last paragraph). The results reported in Noguchi are even more disappointing in view of the facts that

(a) an elevated temperature permits higher enzyme kinetics (see instant specification at page 4, 2nd full paragraph and page 12, 1st paragraph), and

(b) the very reason for using thermophilic enzymes in Noguchi was to promote production yield over side reactions carried out by normal *E. coli* enzymes, almost all of which "are inactivated in the case of conducting the synthesizing reaction at a high temperature" (see translation of Noguchi, page 24, last paragraph).

Accordingly, since Krenitsky teaches that thermophilic and mesophilic enzymes are both effective, and since Noguchi essentially shows that thermophilic enzymes are not particularly effective, it is surprising that the presently claimed cells achieve up to 1040 and 200 times UdP and PNP activity, respectively, even at 30 °C (specification at page 12, 1st paragraph).

The high level of enzyme activity achieved in the claimed cells is also demonstrated in the specification in the following passage (page 10, lines 8-17 and last paragraph):

> The efficiency of these novel strains, both as producers of the enzymes PNP and UdP and as biocatalysts for the preparation of nucleosides by bioconversion reactions, was compared with a preparation of Enterobacter aerogenes cells cultivated in the presence of inducers because that micro-organism, according to the data available in the literature, has hitherto been regarded as one of the best for catalysing transglycosylation reactions (Utagawa et al., Agric.Biol.Chem. 49, 1053-1058, 1985; Utagawa et al., Agric.Biol.Chem. 49, 2711-2717, 1985).
>
> Applying that test, the enzyme activities of UdP and PNP were measured in the recombinant bacterial strains to which the present invention relates and in the comparison E. aerogenes strain, ... which show that the recombinant strains of the present invention have enzyme activities up to approximately 10-30 times higher than that of the comparison strain cultivated under induction conditions and up to approximately 120-1000 times higher than that of the non-transformed E. coli host strains.

These results demonstrate that even if Krenitsky didn't teach away from the present invention and if, for the sake of argument, one had been motivated to combine Krenitsky with Walton, Hershfield, Bulow and Novagen or to combine Krenitsky with Walton, Hershfield, Bulow and Sambrook, one could not have expected or predicted the high activity levels seen in the presently claimed cells. Specifically, higher levels of UdP and PNP activity was seen from the

claimed host cells, which were not induced, than in induced cells which at the time of the present invention were considered the best for catalyzing such reactions.

For all of the above reasons, it is respectfully submitted that the pending claims are unobvious over any combination of the references cited by the Examiner.

Lastly, the Examiner has rejected claim 33 as being allegedly obvious over Krenitsky in view of Walton, Hershfield, Bulow, and Sambrook and further in view of Noguchi (JP 6-253854).

As claim 33 has been cancelled by way of this amendment, this rejection is moot and Applicants respectfully request its withdrawal.

Rejoinder of Withdrawn Process Claims

The Examiner has withdrawn the currently pending process claims of group II (claims 49-53) from Examination under 37 C.F.R. 1.142(b), but has acknowledged that if a product claim is found allowable, withdrawn process claims depending from or otherwise including all of the limitations of the allowable product claim will be rejoined in accordance with MPEP § 821.04 (see page 2 of Action mailed December 11, 2003). Accordingly, these withdrawn process claims have been amended to correct any informalities and to keep the language of the claims consistent with the currently pending product claims. Rejoinder of any currently withdrawn process claim that depends from or otherwise includes all of the limitations of any allowable product claim in the instant application is earnestly solicited.

CONCLUSION

In view of the accompanying amendments and remarks, reconsideration of this application and allowance of all pending claims and rejoinder of the process claims is respectfully requested. If there are any other issues remaining which the Examiner believes could be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

Dated: September 30, 2004

Respectfully submitted,

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